Abstract

It is one objective of the present invention to obtain reproducible representations of expressed mRNA molecules by exploiting a novel technique that relies on short, single stranded polynucleotide tags. In one preferred embodiment, only one polynucleotide tag is obtained from each mRNA molecule, and relatively simple counting statistics can thus be applied after identification and sampling of the different tags, or a subset of tags being present in the population of representative tags. The tags according to the present invention are preferably single stranded polynucleotide tags obtained by subjecting genetic material derived from a biological sample to at least one site-specific nicking endonuclease capable of i) recognizing a predetermined nucleotide motif comprising complementary nucleotide strands and ii) cleaving only one of said complementary strands in the process of generating the at least one single stranded polynucleotide tag. Accordingly, the present invention demonstrates that nicking endonucleases may advantageously be used for obtaining and isolating ssDNA tags. This novel approach in one embodiment eliminates the occurrence of any linker sequence in the ssDNA tag, and it eliminates the presence of a complementary strand in the isolated polynucleotide tag. The lack of linker sequence in the tag and the lack of any complementary strand serves to reduce the huge complexities associated with the analysis of expressed molecules in a biological sample.